3623-Pos Board B351
Cholesterol-Gpcr (B2AR) Interaction in Lipidic Cubic Phase: Insight from 13C NMR
Deborah L. Gater[1], Olivier Saurel[2], Iordan Iordanov[3], Wei Liu[4], Vadim Cherezov[4], Alain Milon[2].
[1]Khalifa University of Science, Technology & Research, Abu Dhabi, United Arab Emirates, [2]IPBS, Toulouse, France, [3]Department of Medical Biochemistry, Semmelweis University, Tuzolto, Hungary, [4]TSRI, La Jolla, CA, USA.
Heteronuclear saturation transfer difference (STD) in high-resolution magic angle spinning (HR-MAS) NMR spectroscopy was applied to lipidic cubic phase (LCP) samples containing monoolein, cholesterol and a G-protein coupled receptor, the beta$_2$ adrenergic receptor (beta$_2$AR), in order to characterize the cholesterol beta$_2$AR interactions. Previous evidence from thermal denaturation experiments and from the observation of conserved binding sites in crystal structures of the beta$_2$AR suggested that cholesterol interacts with the beta$_2$AR with high specificity.
By analyzing ^{13}C chemical shifts variations, STD intensities and ^{13}C T1 relaxation times over a broad range of cholesterol concentration, we have demonstrated that the cholesterol- beta$_2$AR interaction is real, but of very weak affinity.

3624-Pos Board B352
Quantitative Analysis of Ligand-Induced Supramolecular Clustering of Death Receptor 5 in Jurkat Cells
Andrew K. Lewis, Christopher C. Valley, Anthony R. Braun, Jonathan N. Sachs.
Biomedical Engineering, University of Minnesota - Twin Cities, Minneapolis, MN, USA.
Death receptor 5 is a transmembrane protein belonging to the tumor necrosis factor receptor superfamily. Upon ligand binding, DR5 and several members of its superfamily form supramolecular clusters, often modeled as highly organized lattices wherein receptors are retained in their pre-ligand oligomeric assemblies and crosslinked by ligand into high molecular weight networks. Networks can be visualized by fluorescent confocal microscopy, however there is some disagreement as to whether these punctate features are in fact protein assemblies, membrane microdomains (lipid rafts), or a combination of both. Our data show that while ligand induced DR5 networks do assemble in both the presence and absence of lipid rafts, key differences exist in their ability to initiate apoptotic signaling and in the oligomeric structure of the receptor as it is subsumed within the ligand-receptor network. To identify subtle structural differences between supramolecular networks in the presence and absence of lipid rafts, we have developed a technique to detect individual networks and quantitatively compare them between treatment groups in terms of total ligand bound, relative size, and density.

3625-Pos Board B353
Structural Determinants of Protein Association with Membrane Rafts and Consequences of Raft Mislocalization
Ilya Levental, Kandice Levental, Blanca B. Diaz-Aguilar.
University of Texas Medical School at Houston, Houston, TX, USA.
The organization of metazoan membranes into functional domains is a key feature of their physiology. The lipid raft hypothesis emphasizes the preferential interactions between sterols, sphingolipids, and specific proteins as a central mechanism for the regulation of membrane structure and function; however, experimental limitations in defining raft composition and properties have prevented unequivocal demonstration of their functional relevance. Similarly, the physical bases of protein partitioning into lipid rafts remain to be determined. Giant Plasma Membrane Vesicles (GPMVs) isolated directly from live cells separate into coexisting phases of varying orders, physical properties and compositions, allowing analysis of the structural determinants and functional consequences of raft partitioning. By systematic mutation of the transmembrane domain of an integral membrane protein, we observe a direct, quantitative relationship between the length of a protein's transmembrane domain and its partitioning into the raft phase. Next, we generated a panel of variants possessing a range of raft affinities, and used these to establish a quantitative, functional relationship between raft association and subcellular protein sorting. Plasma membrane (PM) localization was dependent on raft partitioning across the entire panel of unrelated mutants, demonstrating that raft association is necessary and sufficient for PM sorting in the absence of other trafficking signals. Abrogation of raft partitioning led to mis-targeting to late endosomes/lysosomes. These findings identify structural determinants of raft association and validate lipid-driven phase separation as a mechanism for protein sorting in the late secretory pathway in non-polarized cells. We are further developing this platform to define the molecular code for transmembrane protein raft association and have discovered key structural features and residues necessary for raft phase partitioning.

3626-Pos Board B354
Rhodopsin Crowding in Model Lipid Bilayers - Functional Implications
Olivier Soubias, John K. Northup, Kirk G. Hines, Walter E. Teague, Klaus Gawrisch.
NIH, Rockville, MD, USA.
In the rod outer segment disks of the retina, rhodopsin is densely packed in phospholipid bilayers with a high content of polyunsaturated acyl chains. It has been hotly debated if oligomerizationof rhodopsin is a critical step for efficient activation of G-protein. Here, we investigated the effect of rhodopsin density in synthetic membranes on the interaction with its cognate G-protein transducin (G_t). Experiments were conducted at rhodopsin:lipid ratios ranging from 1:4000 to 1:70 with sn_1-stearoyl sn_2-oleoyl phosphatidylcholine (18:0-18:1 PC) model bilayers at ambient temperature.
The amount of metarhodopsin-II (MII) formed after photoactivation was determined by UV-visible spectroscopy. Guanine nucleotide exchange rate measured using labeled GTPγS was used to monitor G_t affinity for activated rhodopsin, the rate of rhodopsin catalyzed G_t activation, and the decay rate of the active photointermediate.
At low rhodopsin density (1:1000 and below), MII concentration was the highest and independent of rhodopsin concentration. Increasing rhodopsin packing density correlated with a shift in the metarhodopsin-I (MI)/MII equilibrium towards MI. After photoactivation, rhodopsin decayed at two different rates ($t_{1/2}$ ~ 3 min and > 60 min) and the proportion of the fast decaying photointermediate decreased with increasing rhodopsin density. Finally, MII-catalyzed GDP-GTPγS nucleotide exchange rates for G_t were crucially affected by rhodopsin density. The enzymatic power of rhodopsin (Vmax/Km for Gt) was higher by 2 orders of magnitude at low rhodopsin density as compared to high rhodopsin density.
Our results suggest that, in model membranes, rhodopsin exists as an equilibrium between at least two populations: monomeric at low rhodopsin density with rapid decay and high catalytic efficiency and oligomeric at high rhodopsin density with slow decay and inefficient catalysis of nucleotide exchange.

3627-Pos Board B355
Investigation of Lipid Bilayer Effects on Rhodopsin Activation using UV-Visible and Ftir Spectroscopy
Udeep Chawla[1], Blake Mertz[2], Eglof Ritter[3], Franz Bartl[3], Michael F. Brown[1,4].
[1]Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA, [2]Chemistry, West Virginia University, Morgantown, WV, USA, [3]Institut für Medizinische Physik und Biophysik, Charité - Universitätsmedizin, Berlin, Germany, [4]Physics, University of Arizona, Tucson, AZ, USA.
G-protein-coupled receptors (GPCRs) comprise almost 50% of pharmaceutical drug targets and play crucial roles in signal transduction for a number of physiological processes. Upon photoactivation, rhodopsin undergoes a series of conformational changes leading to visual perception. An ensemble of activated Meta II states is in equilibrium with the inactive precursor Meta I [1]. Lipid bilayer composition and its interaction with membrane proteins govern the ensemble activation mechanism (EAM) as predicted by the flexible surface model (FSM). The FSM describes the balance between intrinsic monolayer curvature and lipid-protein hydrophobic interactions, leading to the elastic coupling of lipids and membrane proteins [2]. Effects of temperature and pH were analyzed for rhodopsin reconstituted in lipid vesicles using UV-visible and FTIR spectroscopy. Thermodynamic properties were derived by fitting phenomenological Henderson-Hasselbalch functions to their respective pH titration curves. Mixed-chain POPC membranes backshift rhodopsin towards Meta I, whereas rhodopsin in DOPC favors the active Meta IIa substate. Analysis of the wavelength-dependent distribution of pKa and alkaline endpoints as estimated from FTIR difference spectra reveals an ensemble of substates for each lipid bilayer-rhodopsin system. The presence of multiple activated conformations is a hallmark of the EAM. Our results are in agreement with the FSM, whereby lipids having a negative monolayer curvature favor the active Meta II state, while lipids with zero spontaneous curvature (POPC) favor the inactive Meta I state [3]. The data give additional insight into the entropy-enthalpy balance which drives the structural conformational changes that occur upon rhodopsin photoactivation. Moreover our work provides fundamental insight into the functionality of other GPCR-related proteins in a natural membrane lipid environment. [1] A.V. Struts (2011) PNAS 108, 8263-8268. [2] M.F. Brown (2012) Biochemistry 51, 9782-9795. [3] E. Zaitseva (2010) JACS 132, 4815-4821.